Report of Short Term Scientific Mission (COST Action FP1301 - EuroCoppice)

"Impact of fertilization on trace element content in Hybrid aspen coppice tree rings"

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INTRODUCTION

Coppicing is a very traditional way of forest management. Recently coppice has been re-discovered because of its adaptive ecology, its stability and multiple benefits, notably its protection function, contribution to biodiversity and as a source of renewable bioenergy. Traditional coppice management is often combined with special ownership and user rights regimes (e.g. commons) and this governance regime may be also an interesting alternative for small scale forestry and/or “modern” short rotation coppice (SRC) which is established on former agriculture land.

Plantations characterized by high yields such as short rotation forestry (SRF) are becoming popular worldwide for biomass production and their role acknowledged in the Kyoto Protocol (Calfapietra et al., 2010). In Baltic countries, where forestry has traditionally been oriented to long rotation periods (commonly 50-120 years), SRF with rotation periods less than 30 years is a new silvicultural concept (Tullus et al., 2012). Agroforestry is a unique land use system that intentionally blends perennial vegetation and herbaceous land cover types to enhance crop productivity, profitability, providing wildlife habitat and maintaining biodiversity (Altieri, 1999), enhance soil enrichment (in particular carbon sequestration) and reducing erosion (Lenka et al., 2012), enhance microbial communities in soil (Banerjee *et al.*, 2015) and overall enhance soil, air and water quality in agroecosystems (Jose, 2009; Baah-Acheamfour et al., 2015). Trees in agroforestry systems constitute a significant avenue of organic matter (and nutrient) addition to the soil ecosystem (Haile *et al.,* 2008); Takimoto *et al.*, 2008; Isaac *et al.*, 2011). The most common species utilized for agroforestry plantations in temperate and boreal climates belong to the genera *Populus* and *Salix* (Calfapietra *et al.*, 2010). In past two decades, hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) have extensively been utilized in the phytoremediation of a broad rage of organic and inorganic (heavy metal) contaminants in soils (Mukherjee, 2014; Marmiroli *et al.*, 2013; Valujeva *et al.*, 2016).

The area of the short-rotation plantations of hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) for pulp and energy wood production with approximately 25-year rotation period has increased considerably during the last few decades in Northern Europe on non-used agricultural lands. Hybrid aspen has proven to be one of the most promising species for intensive pulp and biomass production in this region due to its fast growth, cold and pathogen resistance and the continuously improving planting material provided by long-term breeding programs (Lutter *et al.,* 2016). In Latvia, aspens (*Populus* spp.) are accepted as agriculture energy crop with rotation period up to 5 years since 2011 (The Cabinet of Ministers Regulation No. 173, 2011). The changes made in law “On Agriculture and Rural Development“ at January 1, 2015 proposes that fast growing energy wood tree plantations are agriculture crop with maximum 15 years rotation period. But according to regulations on implementation of the Rural development programme and national subsidies for farmers energy wood crops are eligible for area payments only if they are managed as short rotation coppice with up to 5 years rotation period (The Cabinet of Ministers Regulation No. 126, 2015).

Annual growth rings of trees have the potential to record and preserve annually resolved metal concentrations from atmospheric and soil sources, a technique known as dendrochemistry. The content of the chemical elements in annually formed tree rings are controlled by factors such as the cation binding capacity of woody tissue, radial growth rates, transformation of sapwood into heartwood and processes of radial translocation of elements in the tree stem. Atmospheric pollutants, secondary changes in soil chemistry or any other factors stimulating or reducing tree growth, can influence xylem chemistry, reflecting the actual condition of the tree (Danek *et al.*, 2015).

The most popular methods for chemical analysis of wood – inductively coupled plasma mass spectrometry (ICP-MS) and atomic emission spectroscopy (ICP-AES) – are based on element detection from digested wood. These methods offer multi - element and isotope analysis with low detection limits, but relatively large amounts of wood sample are needed. In contrast, an other group of methods are non-destructive, analyzing intact wood cores without digestion; for example, particle – induced X-ray emission, synchrotron radiation X-ray fluorescence analysis, energy dispersive X-ray fluorescence, and secondary ion mass spectrometry. This group also includes the laser ablation (LA) ICP-MS method. An important advantage of these microprobe-based methods for dendrochemistry is the relatively small amount of material required for analysis in comparison with wood digestion methods, which makes them ideal for analyzing tree rings and producing annually - to - seasonally resolved chemical records (Danek *et al.*, 2015). Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a well established method for direct elemental trace and ultratrace analysis on solid samples which allows contamination free ablation (no pretreatment necessary), high spatial resolution (1–100 μm) and low detection limits, which are beneficial, especially in the case of trees with very narrow growthrings. Therefore LA-ICP-MS is currently the only well established technique which allows direct access to the elemental data stored in incrementally grown layers of biological structures. Thus LA-ICP-MS is also widely used in dendrochemical studies to record pollution fluctuations by monitoring trace elements in single tree rings and is highly advantageous to wet digestion procedures (Prohaska *et al.*, 1998).

PURPOSE

Evaluation of impact of soil fertilization with biogas production residues (30 tonnes ha^{-1}), wastewater sludge (10 tonnes$_{DM}$ ha^{-1}) and wood ash (6 tonnes$_{DM}$ ha^{-1}) on trace elements content in Hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) coppice (managed as agroforesty system) tree rings using laser ablation technique that is the most appropriate analytical method to determine trace metals content and gradient among tree rings.

MATERIALS AND METHODS

Hybrid aspen discs collection and preparation

Hybrid aspen discs were sampled in experimental plantation of hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) coppice managed as agroforesty system in central part of Latvia (Figure 1).

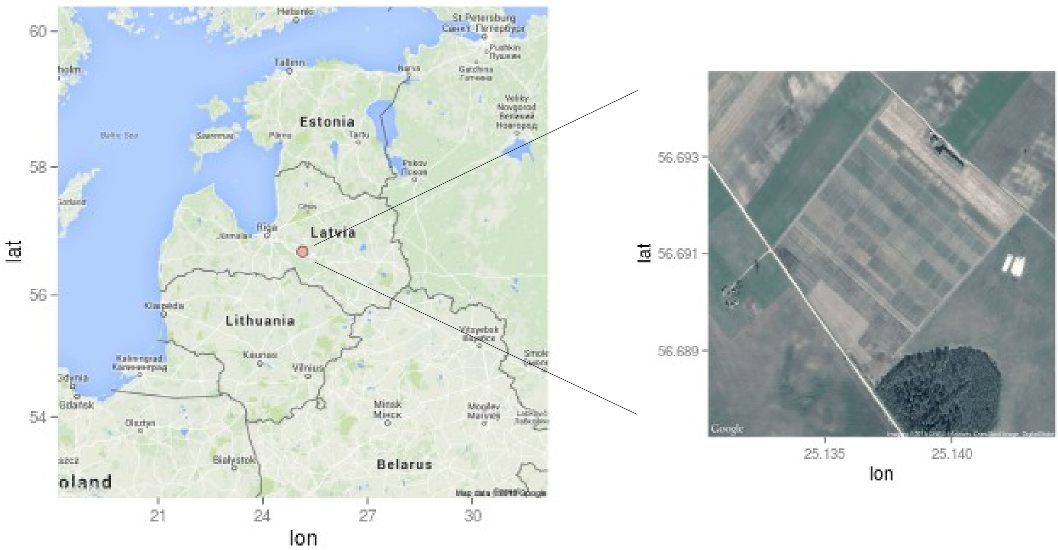


Figure 1: Hybrid aspen coppice experimental plantation in Skriveri (central part of Latvia)

Experimental plot was established on agricultural land in the spring of 2011. Three sample tree (Tab. 1) from four different fertilisation subplots (the size of each plots was – 30 x 24 m) – control (no fertilisation), wastewater sludge (dose: 10 tonnes$_{DM}$ ha^{-1}), wood ash (dose: 6 tonnes$_{DM}$ ha^{-1}) and digestate (fermentation residues from biogas production, dose: 30 tonnes ha^{-1}) were selected and sampled at 0.20 m height (tree ring thickness ~ 2 cm) to all tree rings (6 in total) are represented in the sample.

Table 1: Characterization of sample trees

Sample tree ID	Fertilizer	Tree height, m	Naturally wet stem biomass, kg	Naturally wet branches biomass, kg	$BHD_{1.3}$, mm
A 1	control	8.25	12.10	2.95	63
A 2	wastewater sludge	9.16	16.26	3.88	76
A 3	digestate	9.70	20.94	8.52	83
A 4	wood ash	7.25	12.22	5.18	65
A 5	control	9.14	17.10	4.10	71
A 6	digestate	11.20	32.58	11.66	95
A 7	wood ash	8.93	17.82	8.34	81
A 8	wastewater sludge	9.81	23.10	7.42	89
A 9	control	9.82	17.52	4.88	75
A 10	digestate	10.83	29.84	8.50	94
A 11	wastewater sludge	8.91	20.22	7.86	86
A 12	wood ash	6.53	5.82	1.50	52

Hybrid aspen disks were dried at 70 ºC temperature and divided into particles with max diameter 4 cm (Figure 2).

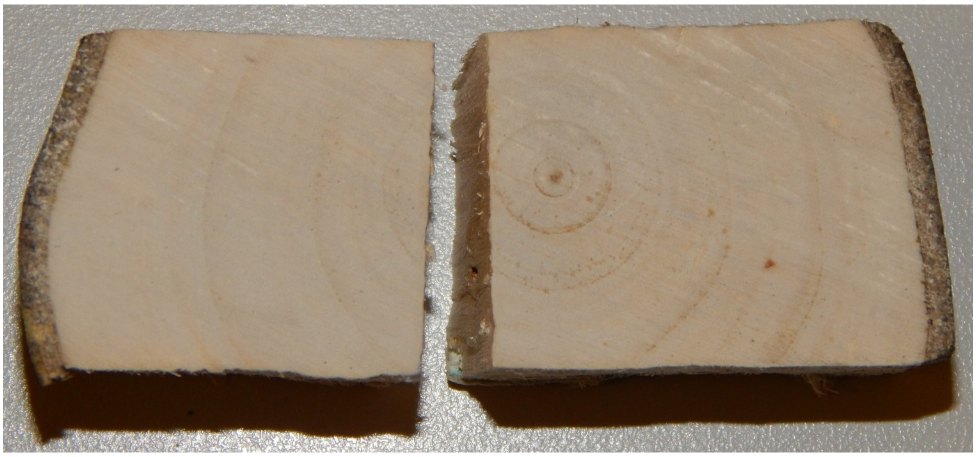


Figure 1: Hybrid aspen disk sample for LA-ICP-MS measurements

LA-ICP-MS measurements

Samples of tree rings were analyzed by laser ablation with mass spectrometry of inductively coupled plasma (LA-ICP-MS, Figure 3). The instrumentation consists of laser ablation system UP213 (NewWave, USA) that generates aerosol of sample from the sample surface and ICP-MS Agilent 7500ce (Agilent Technologies, Japan) used for detection of isotopes. LA-ICP-MS principle scheme is shown in Figure 4. Ablation parameters were optimized with respect to achieve best limit of detection and lateral resolution. Optimized parameters were applied for analysis of all measured samples and are summarized in Table 2. Following isotopes were selected for determination in all samples: ^{13}C, ^{26}Mg, ^{27}Al, ^{31}P, ^{39}K, ^{44}Ca, ^{53}Cr, ^{55}Mn, ^{56}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{111}Cd, ^{202}Hg, ^{208}Pb.

Table 2: Laser ablation parameters

Parameters	Value
laser beam wavelength	213 nm
laser spot size	100 µm
scan speed	70 µm s^{-1}
laser beam fluence	2.5 J cm^{-2}
repetition rate	10 Hz
carrier gas flow	1.0 L He min^{-1}

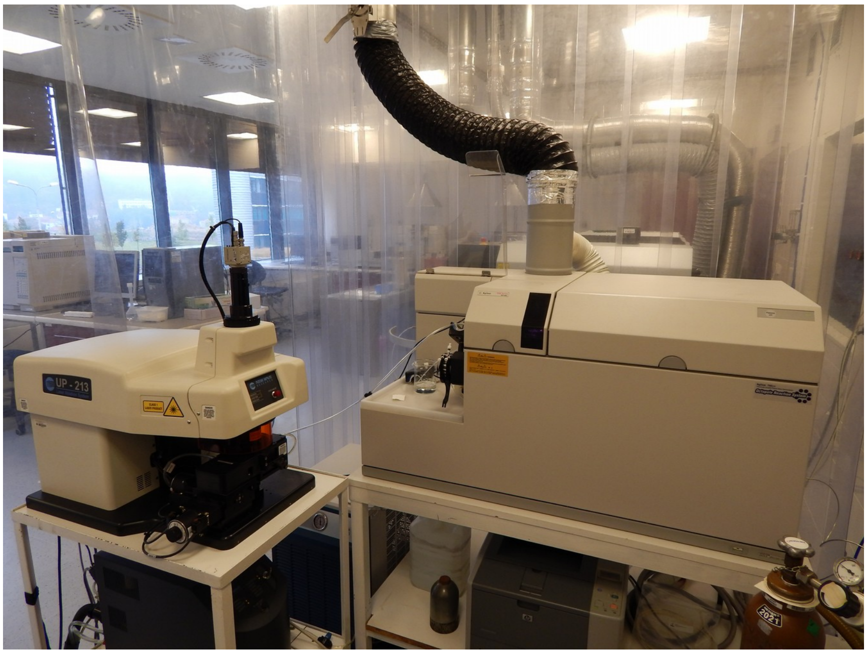


Figure 3: LA-ICP-MS at Masaryk University

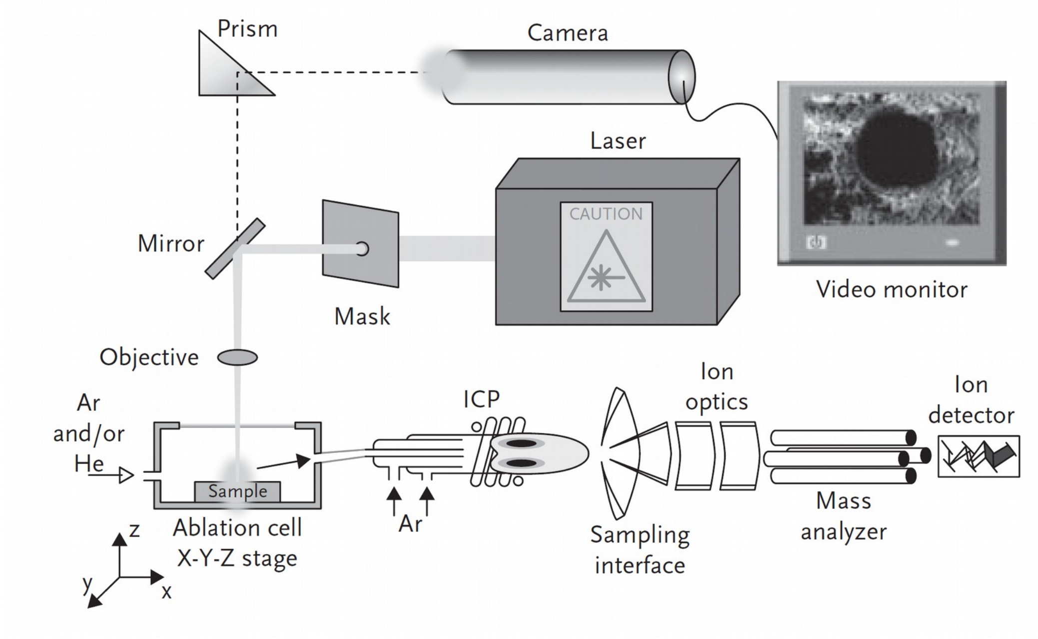


Figure 4: LA-ICP-MS principle scheme

All tree samples were split into 2 or 3 pieces and line scan pattern were used for their ablation. The sample was moved during laser ablation with constant scan speed with straight line trajectory. First ablation pattern went from the center of the tree ring to edge of the sample. Second pattern went perpendicularly to the first one. Design of measurements is shown in Figure 5.

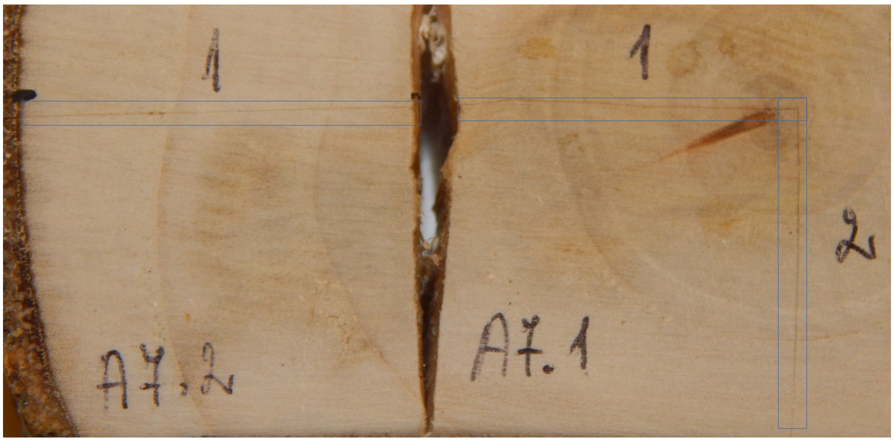


Figure 5: Design of measurements

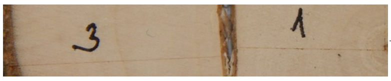
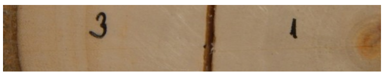
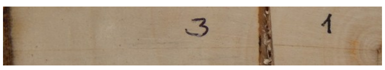
RESULTS

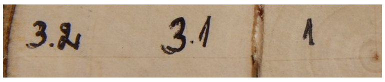
In total, 38 lines for 15 different element isotopes (both macro elements and trace elements) in 12 hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) tree disks were analyzed. In total, 570 data sets of intensities of different isotopes were obtained and initial evaluation of data was done.

The average elements content in unfertilized hybrid aspen wood decreases in the following order: Ca >> K > Mg > Fe > Al > Zn > P > (Mn, Cu) > Pb > (Cr, Ni, Cd). The average elements content in hybrid aspen wood fertilized with wood ash decreases in the following order: Ca >> K > Mg > Al > (Fe, P) > Zn > Mn > Cu > Pb > (Cr, Ni, Cd), but the average elements content in hybrid aspen wood fertilized with digestate or wastewater sludge – Ca >> K > Mg > Fe > (Zn, Al) > (P, Mn) > Cu > Pb > (Cr, Ni, Cd).

The average intensity of trace elements (Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb) in tree rings is summarized in Table 3. The average Hg content in all analyzed samples is below detection limit. Initial evaluation of data sows that the average trace elements content in fertilized hybrid aspen wood are elevated compared to the wood sampled in the control plots (Table 3).

Table 3: The average (mean ± SD) intensity (cps) of trace elements in hybrid aspen tree rings

Sample No	Fertilizer	Picture	Line No	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
1	control		1	4 ± 29	36 ± 39	631 ± 1642	3 ± 12	57 ± 143	196 ± 167	3 ± 2	7 ± 8
2	wastewater sludge		1	14 ± 34	202 ± 63	1231 ± 723	5 ± 10	94 ± 82	622 ± 212	6 ± 2	30 ± 20
			3	11 ± 68	107 ± 42	370 ± 636	0 ± 2	24 ± 36	380 ± 166	4 ± 2	5 ± 10
3	digestate		1	56 ± 79	332 ± 125	2493 ± 2541	18 ± 43	432 ± 1005	1398 ± 1199	11 ± 15	79 ± 78
			3	8 ± 17	135 ± 54	518 ± 607	2 ± 5	31 ± 32	314 ± 181	2 ± 2	10 ± 24
5	control		1	11 ± 24	144 ± 86	854 ± 537	3 ± 4	30 ± 16	252 ± 147	5 ± 4	21 ± 20
			3	8 ± 21	137 ± 96	583 ± 275	1 ± 3	25 ± 15	165 ± 142	3 ± 3	8 ± 17
6	digestate		1	5 ±	142 ±	464 ±	1 ±	36 ±	159 ±	2 ±	8 ±

Sample No	Fertilizer	Picture	Line No	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
				8	61	169	2	23	78	2	14
			3	20 ± 65	154 ± 70	1321 ± 834	4 ± 7	73 ± 74	544 ± 514	5 ± 4	22 ± 15
7	wood ash		A7.1 -1	12 ± 42	9 ± 57	910 ± 463	4 ± 21	51 ± 54	140 ± 74	2 ± 2	18 ± 3
			A7.2 -1	12 ± 13	310 ± 193	1602 ± 2415	4 ± 10	67 ± 37	353 ± 181	4 ± 4	25 ± 25
9	control		1	27 ± 43	236 ± 99	1433 ± 755	11 ± 16	83 ± 92	338 ± 191	6 ± 4	37 ± 14
			3.1 + 3.2	20 ± 34	212 ± 53	1267 ± 564	7 ± 21	63 ± 153	471 ± 153	9 ± 5	43 ± 37
12	wood ash		1	195 ± 525	1857 ± 1007	4580 ± 3925	142 ± 505	451 ± 382	3089 ± 1895	176 ± 130	150 ± 109

Results show tendency to have elevated macro elements (Ca, K, Mg) content in the late or autumn wood compared to the early or spring wood (Figure 6 and Figure 7). In addition, there is a clear correlation between Ca, K and Mg content in the wood.

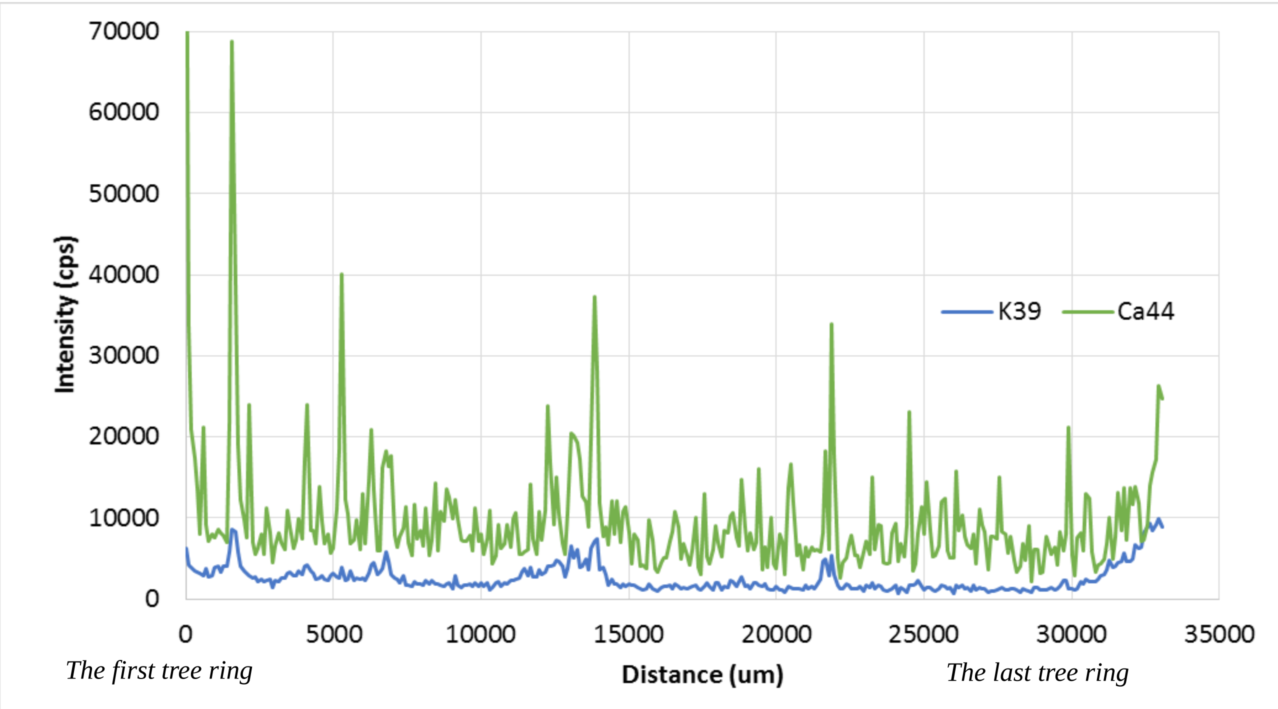


Figure 6: Ca and K intensities across unfertilized hybrid aspen tree rings (sample No 1)

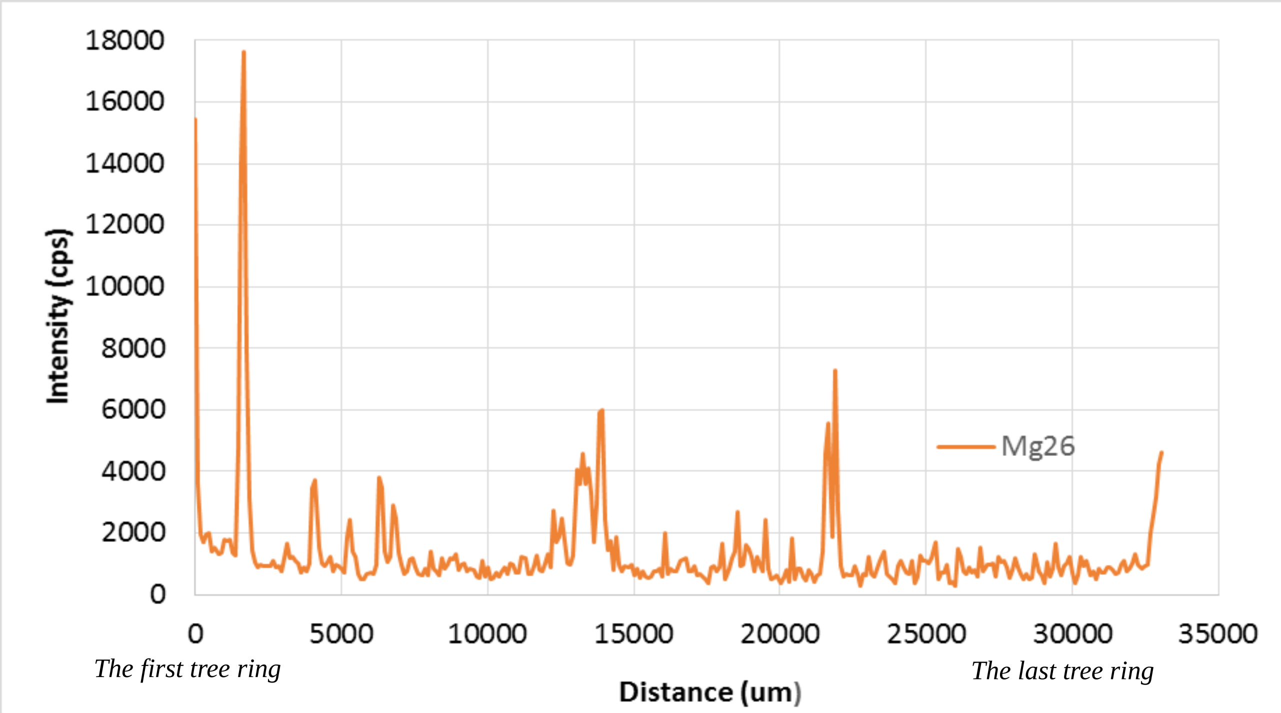


Figure 7: Mg intensity across unfertilized hybrid aspen tree rings (sample No 1)

There is a downward trend of P content in the wood (Figure 8) – P content decreases with increasing distance from pith except maximum peaks of P intensities in the late wood. In the bark, there is elevated P content compared to the tree rings (Figure 9).

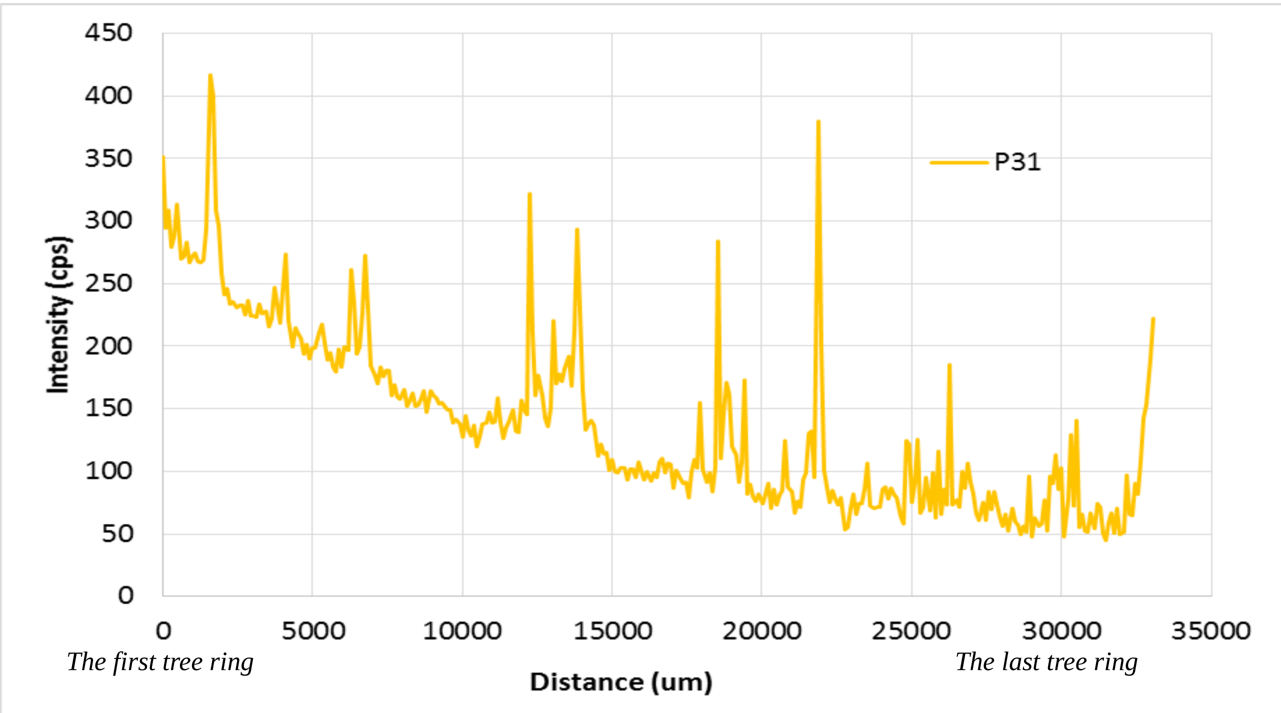


Figure 8: P intensity across unfertilized hybrid aspen tree rings (sample No 1)

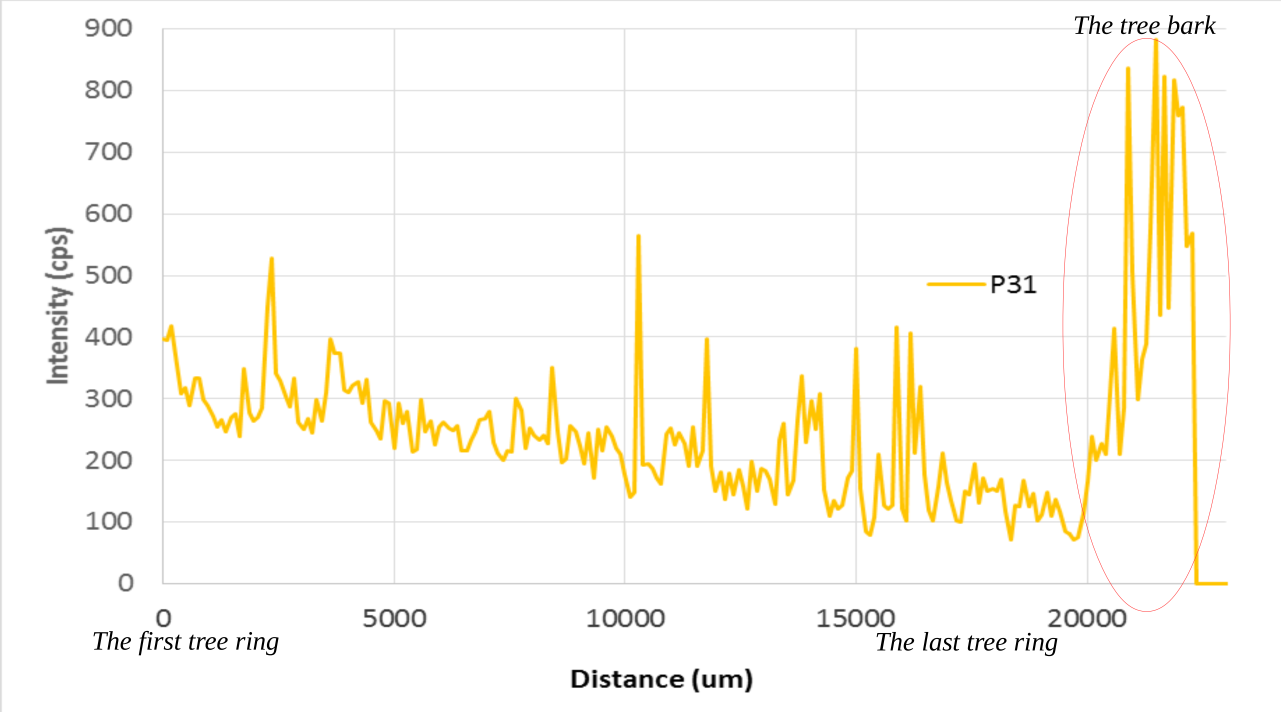


Figure 9: P intensity across hybrid aspen tree rings fertilized with wastewater sludge (sample No 2)

Trace elements (Fe, Cu, Zn, Cd, Pb) intensities across tree rings of unfertilized hybrid aspen (sample No 1) are shown in Figure 10 and Figure 11. There are no clear downward or upward trends in trace elements content across tree rings of hybrid aspen, but an additional data evaluation is necessary to make more detailed conclusions.

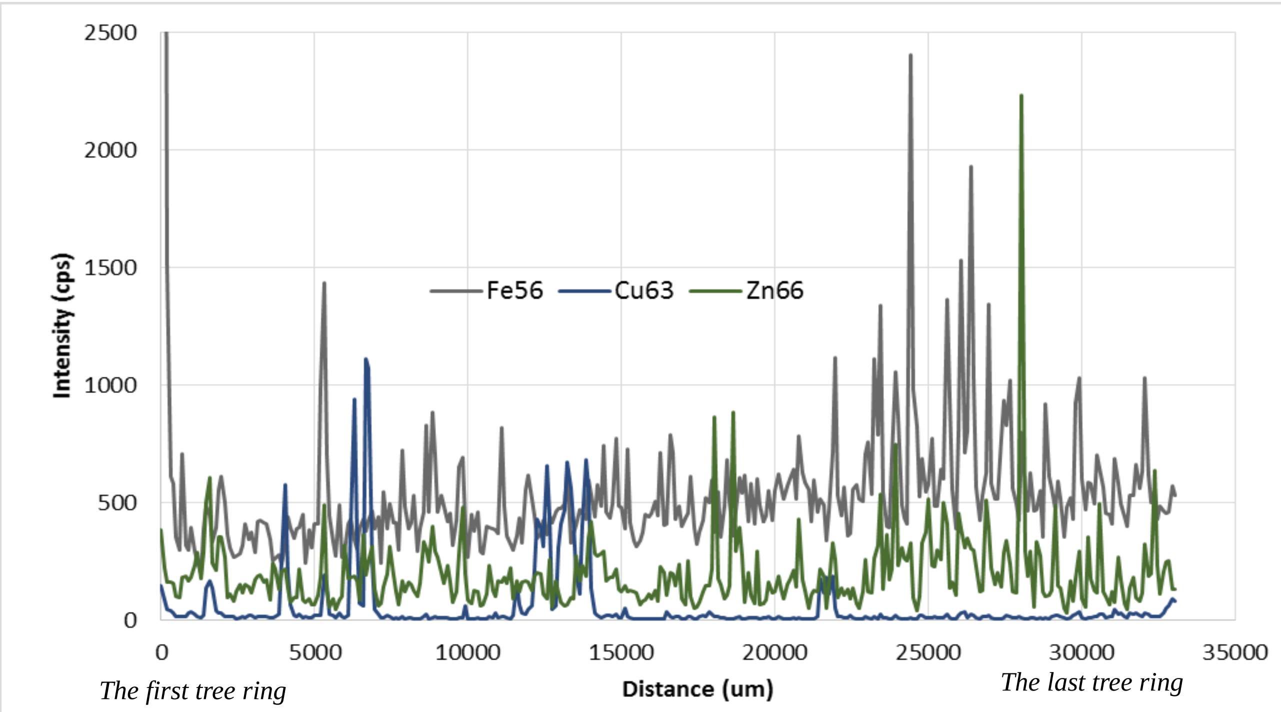


Figure 10: Fe, Cu and Zn intensities across unfertilized hybrid aspen tree rings (sample No 1)

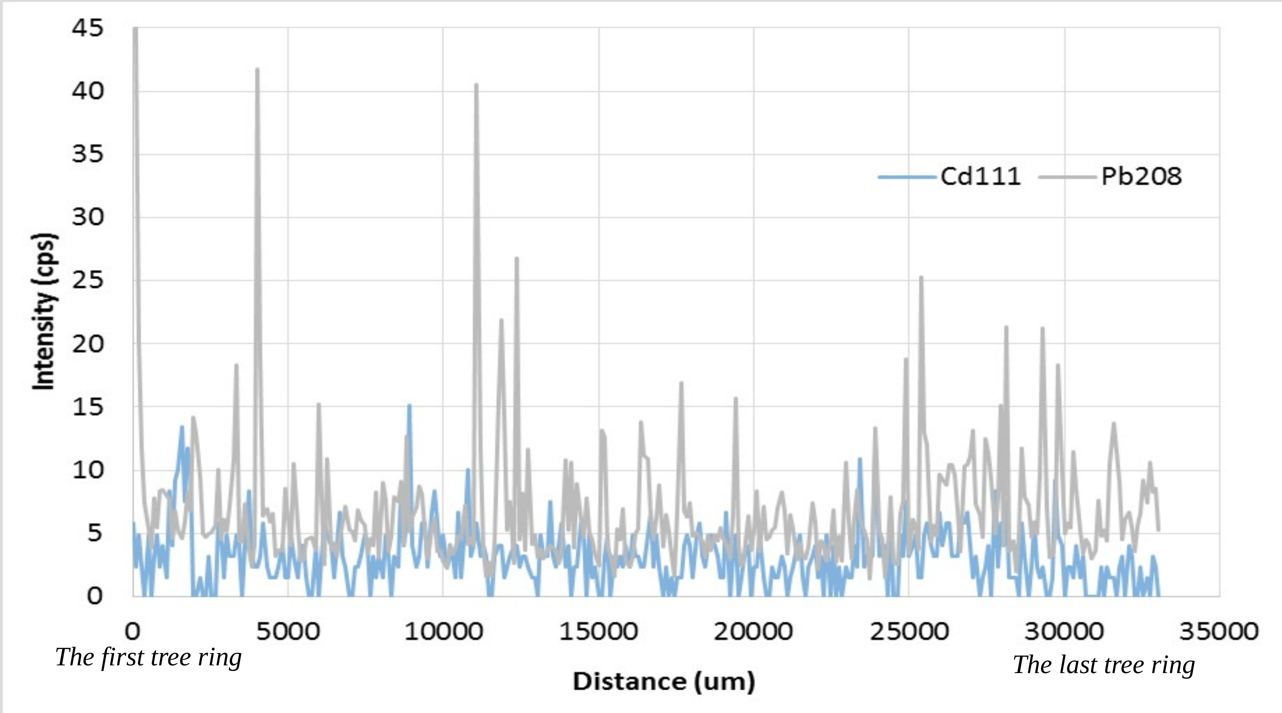
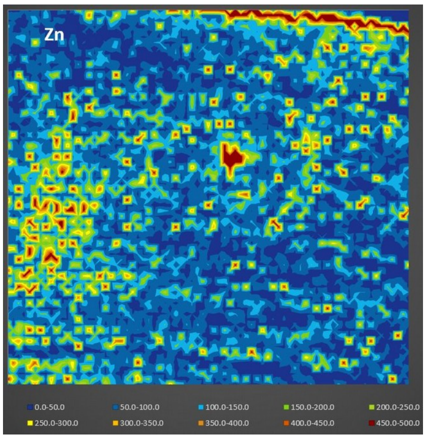


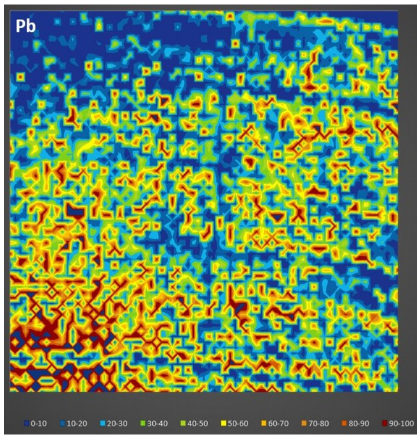
Figure 11: Cd and Pb intensities across unfertilized hybrid aspen tree rings (sample No 1)

Maps of intensities of trace elements in the heartwood of hybrid aspen sample No 4 (fertilized with wood ash) are shown in Figure 12. There is a higher content of several trace elements (Zn, Mn, Cd) in the pith and in the late wood (Mn, Zn) of hybrid aspen.

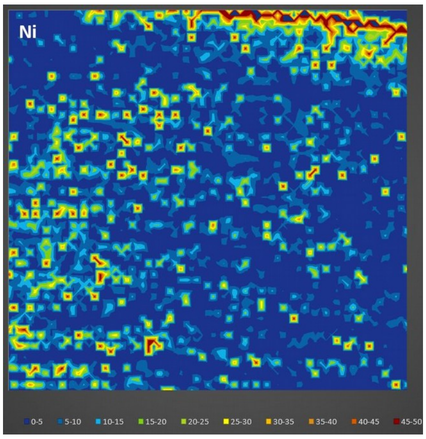
Sample tree No 4



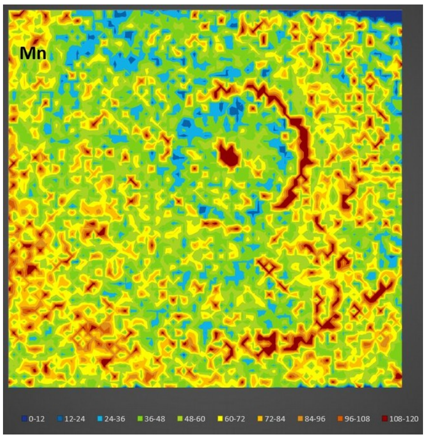
Zn intensity



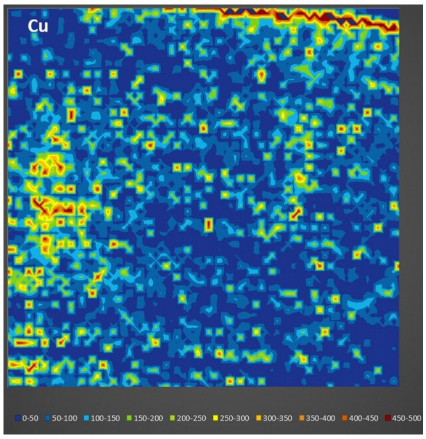
Pb intensity



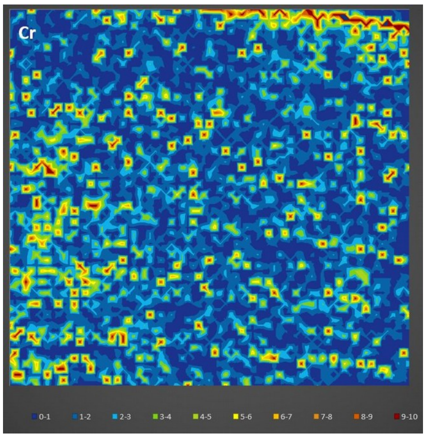
Ni intensity



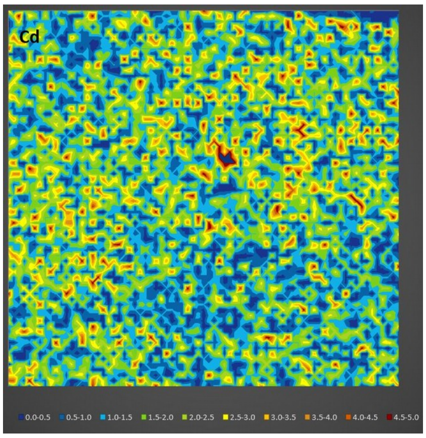
Mn intensity



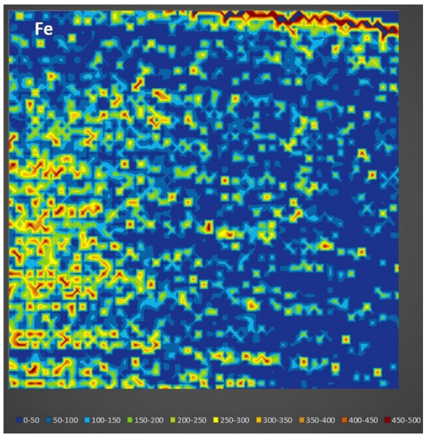
Cu intensity



Cr intensity



Cd intensity



Fe intensity

Figure 12: Maps of intensities of trace elements in the heartwood of hybrid aspen sample No 4

CONCLUSIONS

- Hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) is a sensitive biomonitor to record enhanced heavy metals content in the soil. Results of the study indicate a higher content of trace elements in the wood of fertilized hybrid aspen compared to wood of unfertilized hybrid aspen. Hybrid aspen coppices are an appropriate land use management system for phytoremediation of heavy-metal-contaminated soils purposes.
- Results show that there is a high variation of macro and trace elements content across tree rings with a significant peaks of intensities in the tree pith and in the late wood of hybrid aspen.
- Laser ablation inductively coupled plasma mass spectrometry is an appropriate method to investigate both macroelements and trace elements variation across tree rings as well as to evaluate accumulation potential of trace elements by different tree species.
- Evaluation of the results of the study will be continued to write a research paper for publication and make a more detailed conclusions.

Signed for acceptance

The host – Dr. Viktor Kanicky

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